Redescription of *Brooksiella praeputialis* and *Goezeella siluri* (Eucestoda: Proteocephalidea), parasites of *Cetopsis coecutiens* (Siluriformes) from the Amazon and proposition of *Goezeella danbrooksi* sp. n.

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Redescription of *Brooksiella praeputialis* and *Goezeella siluri* (Eucestoda: Proteocephalidea), parasites of *Cetopsis coecutiens* (Siluriformes) from the Amazon, and proposition of *Goezeella danbrooksi* sp. n. - *Brooksiella praeputialis* (Rego, Santos & Silva, 1974) and *Goezeella siluri* Fuhrmann, 1916 from the Amazonian siluriform fish *Cetopsis coecutiens* (Siluriformes: Cetopsidae) are redescribed. *Goezeella piramutab* Woodland, 1933 is considered as a synonym of *Goezeella siluri*. *Goezeella siluri* sensu Brooks & Rasmussen, 1984 becomes *Goezeella danbrooksi* sp. n., differing from *G. siluri* by the vitelline follicles position (only dorsal, not lateral), size (much smaller in type material) and shape (slightly wider posteriorly); by the position of vaginal sphincter (which is terminal in *G. siluri*) and by the number of testes.

**Key-words:** Eucestoda - Monticelliidae - *Brooksiella praeputialis* - *Goezeella siluri* - *Goezeella danbrooksi* sp. n. - catfish parasites - taxonomy.

### INTRODUCTION

Two proteocephalidean tapeworms, *Goezeella siluri* Fuhrmann, 1916 and *Brooksiella praeputialis* (Rego, Santos & Silva, 1974) have been described from the "candiru", *Cetopsis coecutiens* (Cetopsidae) from the Amazon River. During a field expedition to Amazonia conducted by the Natural History Museum, Geneva (MHNG) and the Institute Oswaldo Cruz (IOC), we were able to collect both cestodes species in excellent condition. Due to some confusion in citations or redescription of both species (Woodland, 1933; Freze, 1965; Rego *et al.*, 1974; Rego, 1975), we give here a brief redescription of *Brooksiella praeputialis* and *Goezeella siluri*, and clarify a number of issues linked to the synonymy of these taxa.

#### MATERIAL AND METHODS

The hosts were collected by fishermen from the Amazon river near Itacoatiara, Brazil, in 1992 and 1995; they were dissected and examined for parasites immediately after death. The gut was dissected along its entire length. The worms were fixed immediately after dissection with hot 4% neutral formaldehyde solution, stained with Mayer's hydrochloric carmine solution, dehydrated in an ethanol series, cleared with eugenol (clove oil) and mounted in Canada balsam. Pieces of the strobila were embedded in paraffin wax, cross sectionned (thickness 12-15  $\mu m$ ), stained with Weigert's hematoxylin and counterstained with 1% eosin B (de Chambrier, 2001). Eggs were studied in distilled water. All measurements are given in micrometers ( $\mu m$ ) unless otherwise stated.

Abbreviations used in descriptions are as follows: x = mean, n = number of measurements, CV = coefficient of variability, OV = ovary width versus proglottis width ratio (in %), PP = position of genital pore (cirrus pore) as % of proglottis length, PC = cirrus-pouch length versus proglottis width ratio (in %). CHIOC = the Helminthological Collection of the Institute Oswaldo Cruz; MHNG = Natural History Museum, Geneva, INVE = Geneva Museum Invertebrate Collection, USNM = U.S. National Museum.

#### RESULTS

# Brooksiella praeputialis (Rego, Santos & Silva)

Figs 1-5, 9-10

Amphoteromorphus praeputialis Rego, Santos & Silva, 1974: 200. Amphoteromorphus praeputialis; Brooks & Rasmussen, 1984: 751. Brooksiella praeputialis; Rego, Chubb & Pavanelli, 1999: 354.

Type-host: Cetopsis coecutiens Spix & Agassiz (Siluriformes: Cetopsidae); vernacular name : Candiru.

Other host: Pseudocetopsis othonops Eigenmann (Siluriformes: Cetopsidae).

Materiel studied: Brooksiella praeputialis (Rego, Santos & Silva, 1974), syntypes CHIOC 26.421, 31.047a-d, 31.048 a-c and 26.524.

Other material: Itacoatiara, Amazonas State, Brazil, MHNG INVE 19357 (14.09.1992); INVE 21875, 21876 (07.10.1995); INVE 22059, 22088, 21994, 21995, 21996 (16.10.1995).

Site of infestation: Intestine.

Type locality: Rio Amazonas, Maicuru, Pará State, Brazil.

Distribution: Amazon River.

## REDESCRIPTION (based on 10 specimens)

Proteocephalidae, Zygobothriinae. Cestodes up to 12-22 mm long, 1,785 wide, with numerous transverse grooves in immature and mature proglottides, less marked in gravid proglottides. Strobila acraspedote, anapolytic, consisting of 46 to 51 (n = 7) proglottides: 25-36 immature (up to appearance of spermatozoa in vas deferens), 2-4 mature (up to appearance of eggs in uterus), 7-15 pregravid (up to appearance of hooks in oncospheres), and up to 8 gravid proglottides. Proliferation zone very short.

Immature, mature, pregravid proglottides wider than long (Fig. 3), gravid proglottides slightly wider than long or longer than wide. Metascolex slightly conical 910-1370 (x = 1145, n = 10) in diameter, much wider than neck (Figs 1, 9-10), with antero-laterally directed, embedded, uniloculate suckers, 275-395 (x = 325, n = 40, CV

= 9%) in diameter. Suckers with a well-developed circular musculature situated in upper marginal region (Fig. 1). External surface of metascolex bearing numerous wrinkles (Figs 9-10).

Internal longitudinal musculature weakly developed, represented by fine bundles of separated muscular fibres (Figs 4-5) forming anastomoses. Osmoregulatory canals overlapping vitelline follicles and testes (Figs 3-5). Ventral canals wider than dorsals. Presence of a posterior transverse canal and of secondary canals situated posteriorly and ending laterally beneath the tegument. Within scolex, canals form a dense network (Fig. 1).

Testes medullary, forming 2 compact fields in two or three layers, not overlapping cirrus sac, vagina and vas deferens (Fig. 3), numbering 193-292 (x = 244, n = 5, CV = 19%); aporal testes 98-150 in number (x = 123); 17-25 preporal testes (x = 21); 141 postporal testes 75-122 (x = 98). Testes spherical to oval, 45-60 in diameter, representing 2.5 - 4% of proglottis width. Testes reaching laterally almost the margin of proglottides, but not reaching not the posterior one (Fig. 3), overlapping vitelline follicles, present also in gravid proglottides.

Cirrus sac elongate to pyriform, with tapered distal part, thick-walled, 250-385 long and wide (Fig. 3); PC 15-23% (x = 19%, n = 36, CV = 11%). Cirrus up to 65% of cirrus pouch length. Vas deferens strongly coiled, situated between proximal part of cirrus sac and midline of proglottides, occupying up to 32% of proglottis width, wider in its basal part (Fig. 3).

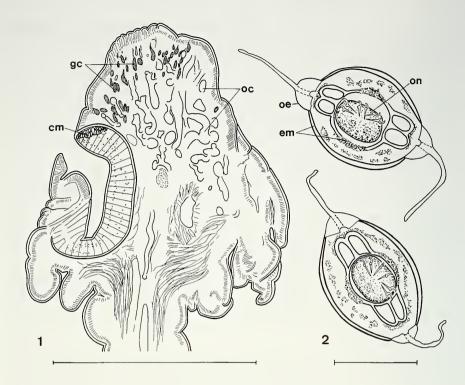
Genital atrium deep, pores alternating irregularly, situated anteriorly; PP = 12-26% (x = 18%, n = 24). Genital ducts passing between osmoregulatory canals.

Ovary medullary, bilobate, highly follicular in pre-gravid proglottides (Figs 3-4), with numerous dorsal and ventral outgrowths scarcely reaching dorsal cortex (Fig. 4), 880-1210 wide, OV =55-69 % (x = 59%; n = 34; CV = 7%). Mehlis' glands 100-150 in diameter (Fig. 3).

Vitelline follicles cortical, arranged in two lateral ventral field near margins of proglottides, more numerous posteriorly, occupying almost all proglottis length, not interrupted at level of cirrus sac ventrally, with some isolated follicles dorsal to cirrus sac. Vitelline fields of ventral follicles overlapping testes (Figs 3-5).

Vaginal canal forming small seminal receptacle antero-dorsal to ovarian isthmus, terminal part of vaginal canal (pars copulatrix vaginae) muscular (Fig. 3). Vagina anterior 26(%) or posterior 74(%) (n = 126) to cirrus-sac. Vaginal canal strongly curved before reaching seminal receptacle.

Primordium of uterine stem medullary, present in immature proglottides. Formation of uterus of type 1 (see de Chambrier *et al.*, 2004b): in immature proglottides, uterine stem straight, occupying entire length of proglottis, formed by longitudinal thick column of chromophilic cells along midline of proglottides. Lumen of uterus appearing in first mature proglottides (Figs 3-5); diverticula (lateral branches) formed before first eggs appear in uterine stem. In pregravid proglottides, eggs completely filling uterine stem and thick-walled diverticula. In gravid proglottides, diverticula occupying up to 62 % of proglottis width; 9-17 (n = 26) medullary lateral branches on each side. Longitudinal ventral uterine opening observable in some terminal proglottides.



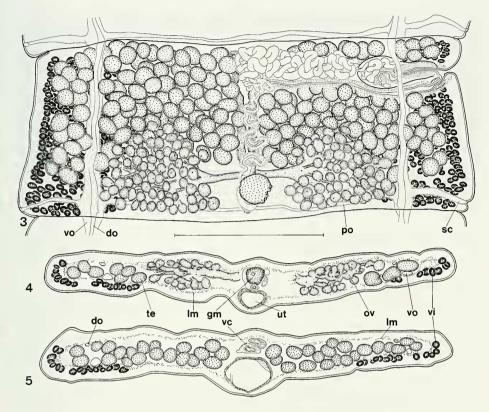
Figs 1-2

Brooksiella praeputialis (Rego, Santos & Silva, 1974), INVE 22059. 1. Frontal section of the scolex, showing the concentration of gland(?) cells and dense network of osmoregulatory canals beneath the apical part. Note also the muscular fibres situated in the anterior part of the sucker. 2. Eggs drawn in distilled water. Abbreviations: cm, internal circular musculature; em, embryophore; gc, gland cells; oc, osmoregulatory canals; oe, outer envelope; on, oncosphere. Scalebars:  $1 = 500 \ \mu m$ ;  $2 = 20 \ \mu m$ .

Eggs with two polar projections 15- 20 long (Fig. 2). Embryophore spherical, bilayered, 25-29 x 22-24; internal layer containing granular material and paired polar chambers, 22-26 x 13-16; oncosphere spherical to oval, 10-13 in diameter, with 3 pairs of hooks, 4-5 long (Fig. 2).

#### REMARKS

Brooks & Rasmussen (1984) commented on the confusion of figures by Rego *et al.* (1974). We re-examined the original material and agree with Brooks & Rasmussen: figures 23, 24, 26 and 27 of Rego *et al.* (1974) are of *B. praeputialis* (No CHIOC 31047a, 31047d respectively) and not *Monticellia siluri* as stated. Figures 30-32 (CHIOC 31048a) and figure 33 (CHIOC 31048c) correspond to the syntype material of *B. praeputialis*.



Figs 3-5

*Brooksiella praeputialis* (Rego, Santos & Silva, 1974). 3. INVE 21996, mature proglottis, dorsal view. 4-5. INVE 22059, pregravid proglottis, cross sections at level of ovary and at level of middle of the proglottis. Note the follicular structure of ovary and the lateral and ventral position of vitelline follicles. *Abbreviations*: do: dorsal osmoregulatory canal; gm, Mehlis glands; lm, internal longitudinal musculature; ov: ovary: po: transverse osmoregulatory canal; sc: secondary canal; te: testes; ut: uterus; vc: vaginal canal; vi: vitellaria; vo: ventral osmoregulatory canal. Scale-bar: 500 μm.

## Goezeella siluri Fuhrmann

Figs 6-8

Goezeella siluri Fuhrmann, 1916: 386.

Goezeella piramutab Woodland, 1933: 488, syn. n., see discussion below.

Monticellia siluri; Woodland, 1925: 714.

Goezeella siluri; Freze, 1965: 508.

Monticellia siluri; Rego, Santos & Silva, 1974: 195.

Goezeella siluri; Brooks & Deardorff, 1980: 15.

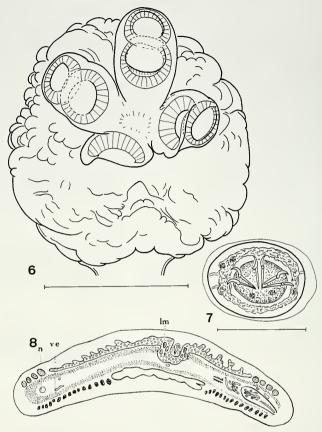
Goezeella siluri; Rego, Chubb & Pavanelli, 1999: 348.

[Not Goezeella siluri; Brooks & Deardorff, 1980: 15]. See Goezeella danbrooksi sp. n., described below.

[Not Goezeella siluri; Brooks & Rasmussen, 1984: 750]. See discussion below.

Type-host: Cetopsis coecutiens (Siluriformes: Cetopsidae).

Other host: Pinirampus pirinampu (Spix & Agassiz) (Siluriformes: Pimelodidae).



Figs 6-8

Goezeella siluri Fuhrmann, 1916. 6. Scolex, type material showing the biloculate suckers. 7. INVE 19858, egg drawn in distilled water. 8. Cross section at level of ovary, showing the ventral position of vitelline follicles, after Fuhrmann (1931, Fig. 275). Abbreviations (according to Fuhrmann): Im, internal longitudinal musculature; n, longitudinal nerve; ve, ventral osmoregulatory canal. Scale-bars:  $6 = 1000 \, \mu m$ :  $7 = 20 \, \mu m$  ( $8 = no \, scale$ ).

Materiel studied: Goezeella siluri, syntype material, 2 whole mount slides, 14 cross-sections slides, Coll. Institut de Zoologie, Neuchâtel. Other material: Itacoatiara, Amazonas State, Brazil, MHNG INVE 19858, 13.09.1992.

Site of infestation: Intestine.
Type locality: "Amazonas", Brazil.
Distribution: Amazon River.

### DESCRIPTION

Strobila somewhat contracted, 77 mm long, 2025 in width. About 47 immature, 10 mature and 143 (pregravid and gravid) proglottides. Scolex with metascolex, 1825 in diameter, with biloculated suckers 435 long and 300 width (Fig. 6). Internal longitudinal musculature developed, represented by fine bundles of separated muscular fibres (Fig. 8) forming anastomoses.





Figs 9-10

*Brooksiella praeputialis* (Rego, Santos & Silva, 1974), INVE 22088. Scanning electron micrographs. 9. Scolex, apical view. 10. Scolex, lateral view. Scale-bar: 100 μm.

About 380-433 small testes in one cortical dorsal field and in one layer. Deep genital pore irregularly alternating, anterior, PP = 10-16%. Cirrus pouch elongated to pyriform, PC = 13-17% (x = 14%, n = 9), cirrus occupying between 30-50% of cirrus pouch length. Vagina always anterior to cirrus pouch (n = 36), with powerful muscular terminal sphincter. Ovary cortical, bilobate, follicular, with numerous dorsal outgrowths, OV = 75-79%.

Ventral cortical vitelline follicles lateral, occupying large triangular field, wider posteriorly, overlapping ovary and cirrus pouch, running from anterior to posterior margin of proglottis. Ventral cortical uterus of type 2 (see de Chambrier *et al.*, 2004b, for definition), with 14-21 thin digitate diverticula with thick walls. Eggs oval (in whole mounts), embryophore bilayered, about 20 x 16; oncosphere spherical to oval, about 10 in diameter (Fig. 7).

## Goezeella danbrooksi sp. n.

Goezeella siluri; Brooks & Deardorff, 1980: 15. [Not Goezeella siluri Fuhrmann, 1916: 386].

Type-host: Ageneiosus cancanus (Siluriformes: Ageneiosidae).

*Holotype* (examined): 1 unique specimen with some proglottides cut in serial sections, USNM Helminthological collection No. 74498 (2 slides), No. 74544, (19 slides, serial sections). In our knowledge, no other material of this species cited in literature.

Site of infestation: Small intestine near juncture of stomach and intestine.

Type locality: Magdalena River, vicinity of San Cristobal, Bolivar Province, Colombia.

#### REMARKS

Brooks & Deardorff (1980) studied, in a detailed description, *Goezeella siluri* in *Ageneiosus cancanus* Steindachner from Columbia. De Chambrier & Vaucher (1999, p. 184) noted that the species described by these authors apparently did not belong to *G. siluri*. When looking at figures 2 to 5 of Brooks & Deardorff (1980), we observed that their material differed from the type material of *G. siluri* (see Fig. 8,

present work) in several aspects: the position of the vitelline follicles (only dorsal, not lateral), relative size (much smaller in type material), and shape (slightly wider posteriorly, see Fuhrmann, 1916, Fig. 4). *Goezeella siluri* sensu Brooks & Deardorff also differs in the position of vaginal sphincter (terminal in *G. siluri*), by the size of the cirrus / cirrus pouch ratio, and by the number of testes (183-380 versus 380-433). Furthermore, the taxon described by Brooks & Deardorff parasitizes a different family of hosts (Ageneiosidae).

In our opinion, *Goezeella siluri* sensu Brooks & Deardorff represents a distinct species and we name it *Goezeella danbrooksi* sp. n. in honour of Prof. Daniel R. Brooks from Toronto, Canada.

# DISCUSSION

Fuhrmann (1916) in his description of *G. siluri*, observed oval eggs without polar projections. Woodland (1933) in his expedition in Amazonian river in 1931, found in the type host *Cetopsis coecutiens* (field number Amaz 174) twenty three worms he determined as *G. siluri*. He first thought that he had two different species, as six specimens fitted Fuhrmann's description (thick proglottides, similar transverse sections) but the remaining specimens included much thinner proglottides. He finally decided to consider that all the material belonged to a single species because the "eggs of both types are identical in shape and size". In fact, Woodland observed and drew only those from *B. praeputialis* (his Fig. 18, compare with Fig. 2, present work).

The material described by Woodland (1933) as *Goezeella siluri* (BMNH 1964.12.15.174-183) is in fact a mixture of two species: *G. siluri* Fuhrmann, 1916 with biloculate suckers (Fig. 6, present work) and *Brooksiella praeputialis* (Rego, Santos & Silva, 1974) with uniloculate suckers (Figs 1, 9-10, present work). The shape described by Woodland (1933) as "cobra like" due to the hood shaped neck (Woodland's Fig. 12) corresponds with *Brooksiella praeputialis* (Woodland's plate 30, Figs 10, 11, 12, 14, 17, 18) and the other figures correspond with *Goezeella siluri* (Figs 9, 13, 16). Of his six whole mounted slides, three are a mixture (slides number 1, 3, 6), one is *G. siluri* (slide number 4) and two are *B. praeputialis* (slides number 2, 5). The cross sections numbered G'1-6 and G" 1-3 are of *G. siluri* (Woodland's Fig. 16) and those G 1-2, Gc 1-7 and G" 1-2 correspond to *B. praeputialis* (Woodland's Fig. 17).

In the same paper, Woodland (1933) considered *Goezeella piramutab*, a parasite of *Brachyplatystoma vaillanti*, as a new species. He based his decision to separate *G. piramutab* from *G. siluri* only on the differences on eggs shape and size. He suggested that a complete diagnosis must await the examination of further material. In fact, Woodland equivocally compared eggs from *B. praeputialis* (Woodland's Fig. 18) and eggs of *G. piramutab* (Woodland's Fig. 22). In our opinion, the eggs figured by Woodland (Fig. 22) belong to *Goezeella siluri* (see Fig. 7, present work). The other eggs (Woodland's Fig. 18) are typical of *B. praeputialis* (compare with Fig. 2, present work). As the argument to separate *G. piramutab* from *G. siluri* by their eggs shape and size is no longer valid, we consider *Goezeella piramutab* as a synonym of *Goezeella siluri*.

Brooks & Rasmussen (1984) found a cestode they determined as *B. praeputialis* in a new host, *Pseudocetopsis othonops* in Venezuela but did not give a description.

Judging by their figures (5, 6, 7), we believe that it may correspond to another species : the diameter of scolex is smaller  $(705 \, \mu m \text{ versus } 1145 \, \mu m \text{ in mean})$ , the suckers seem to be deeply embedded and the testes are less numerous according to their figures  $(6-7, p. 751) \, (64-178 \, \text{versus } 205 - 342)$ .

The shape of the ovary of *Brooksiella praeputialis* is particular. It is highly follicular and is similar to that of *Goezeella siluri*. This is an unusual observation for the Proteocephalidea, although this ovarian shape is also present in *Manaosia bracodemoca* Woodland, 1935 and *Electrotaenia malapteruri* (Fritsch, 1886) (see de Chambrier, 2003; de Chambrier *et al.*, 2004a).

Brooksiella praeputialis shows some similarities with the species of Rudol-phiella: a metascolex, a wide band of ventral vitelline follicles, a follicular ovary, type 1 uterine formation and eggs with a similar structure including polar formations and bilayered embryophores. Furthermore, in a recent molecular analysis, two Rudol-phiella species clustered with Brooksiella praeputialis in a strongly supported clade (see de Chambrier et al., 2004b: fig. 1. Rudolphiella possesses cortical vitelline follicles and testes, and medullar ovary and uterus, whereas in Brooksiella only the vitelline follicles are cortical. Both genera belong to two different subfamilies, Rudolphiellinae and Zygobothriinae, which are characterised by a different arrangement of the genital organs relative to the internal longitudinal musculature. However we have recently expressed doubts about the importance of this type of character and have suggested that other characters may prove to be useful in suprageneric classifications of the Proteocephalidea (de Chambrier et al., 2004b).

## **ACKNOWLEDGEMENTS**

The authors thank David I. Gibson and Eileen Harris for loan of material. They also thanks anonymous referees who provided judicious comments on an earlier version of this manuscript, Jean Wuest (Geneva) for the SEM photography, Gilles Roth (Geneva) for finalising the drawings and the fishermen in Itacoatiara, whose collaboration in providing freshwater fishes was greatly appreciated.

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